biosynthesis of an enzyme involved in regulating cytoplasmic cleavage.

In view of the great emphasis placed on shape and size of autospores in species classification, the variation limits of each morphological characteristic should be carefully determined before a species concept is formed. This variant would undoubtedly have been classified as a new species, if it had not arisen spontaneously in a stock culture of *P. wickerhamii*.

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30 March 1967

Primordial Germ Cells in Blood Smears from Chick Embryos

Abstract. *The feasibility of studying avian primordial germ cells in blood smears has been demonstrated. Blood smears prepared from chick embryos of stages 13 to 15 (48 to 55 hours) contained primordial germ cells, which were revealed by the periodic acid-Schiff reaction. The presence of glycogen in the cytoplasm of the primordial germ cells facilitated their selective identification.*

Primordial germ cells (PGC's) have been consistently reported in the blood vessels of early chick embryos because the vascular system provides the chief, if not only, route for the migration of these cells from their place of origin, the extraembryonic germinal crescent, to the site of their ultimate disposition, the gonadal ridge (*1, 2*). Although the size and morphology of the PGC's are characteristic, cytochemical methods have had to be employed to demonstrate these cells, particularly in extragonadal areas. In contrast to mammalian PGC's, which possess sufficient quantities of histochemically demonstrable alkaline phosphatase activity to permit their selective identification (*3, 4*), chick PGC's cannot be distinguished by this technique, owing to the uniform distribution of enzymatic activity among all embryonic cells (*3*). Instead, the chick PGC's have been found to contain abundant cytoplasmic deposits of glycogen which greatly facilitate their recognition in tissue sections subjected to the periodic acid-Schiff (PAS) reaction. Using this technique, Meyer (*2*) observed that the maximum numbers of PGC's are present in the intraembryonic, vascular system during stages 13 (19 somites; 48 to 52 hours) to 15 (24 to 27 somites; 50 to 55 hours) (*5*). Therefore, blood smears prepared from embryos at these critical stages should provide an excellent means for further exploration of the PGC's provided, of course, that they can be reliably distinguished from the other blood elements. Such has not been the case with routine blood stains (*6, 7*).

Blood was removed from the dorsal aortas of chick embryos between stages 13 and 15 with the use of micropipettes according to the method outlined by Lucas and Jamroz (*6*). One half of the smears were dried and the other half were fixed in Gendre's fluid (for glycogen localization). Under the phase microscope, the PGC's were identified by their large size (related to that of the blood cells), their eccentric nuclei, and the presence of refractile vacuoles within their cytoplasm. This cellular appearance closely resembled that of living PGC's observed in squash preparations of whole embryos (Fig. 1). In preparations subjected to the PAS reaction, some of the cytoplasmic vacuoles became intensely magenta-colored; others remained unreactive (Fig. 2). We subsequently confirmed the PAS-positive material to be glycogen by subjecting control smears to malt diastase (*8*) prior to their treatment with periodic acid. The staining of unfixed, dry smears with oil red O (in propylene glycol) also revealed that the PAS-negative vacuoles were actually sites of lipid material (yolk). Dantschakoff (*9*) adopted this characteristic, among others, to identify the PGC's.

The distribution of the PGC's in the circulating blood of staged chick embryos is shown in Fig. 3. By stage 18 the presence of PGC's in blood smears was rare. A few blood cells displayed a slight diffuse reaction to the PAS technique, but they were easily distinguished from the PGC's by their small size.

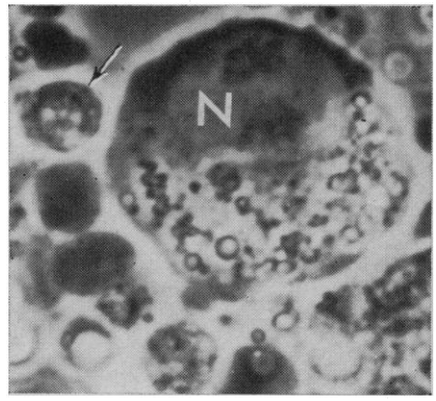

Fig. 1. Phase photomicrograph of living chick PGC in squash preparation of whole embryo, stage 15. Note the large size of the PGC in comparison to that of a blood cell (arrow). *N*, nucleus (× 2000). (Prepared in the laboratory of Dr. R. J. Blandau.)

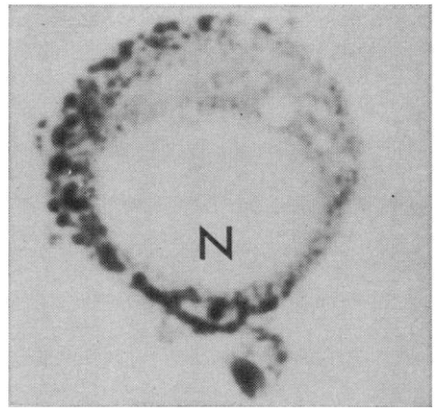

Fig. 2. Photomicrograph of PGC in blood smear at stage 15 stained by the PAS reaction. The dark staining bodies (magenta with the PAS reaction) are glycogen digestible with diastase. The unstained vacuoles represent lipid material. *N*, nucleus.

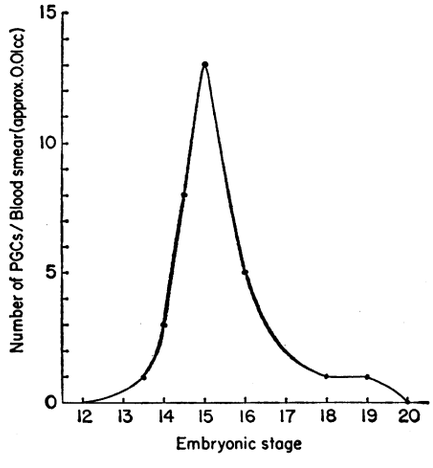

Fig. 3. Distribution of PGC's in the circulating blood of staged chick embryos based on the average of blood smears from five embryos at each stage.

The ease with which chick PGC's can be obtained and identified in blood smears now affords a valuable means for further characterization of these cells.

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21 April 1967

Temperature Effect on Protein Synthesis in a Heat-Synchronized Protozoan Treated with Actinomycin D

Abstract. *Protein synthesis was studied in the ciliated protozoan* Tetrahymena pyriformis *GL after actinomycin D was added to the culture medium. When the temperature rose above that optimum for growth, there were significant reductions in protein synthesis. Lipid biosynthesis under the same conditions was slightly stimulated, an indication that the effect was not due to an underlying reduction in energy sources. The phenomenon appears to be unique to the protein synthesizing system. Correlation with previous data suggests that it is due to nontranslational destruction of template RNA.*

To a striking degree, the stability of labeled RNA depends upon temperature in *Tetrahymena pyriformis* GL (*1*), a ciliated protozoan that can be brought into division synchrony by a cyclical heat treatment (*2*). Because the exact rate of RNA degradation could not be unambiguously determined at the optimum growth temperature (29°C) we have studied protein synthesis after the addition of actinomycin D in an effort to explore this problem further. When actinomycin D (50 μg/ml) is added to a *Tetrahymena* culture, uptake of uracil stops—and the rate of protein synthesis falls gradually to zero (*1*). Since both RNA (*3*) and protein (*4*) synthesis are required for synchronous division of these organisms, it was important to ascertain whether the unstable RNA fractions included template RNA. Further, it was of interest to see whether the increased rate of degradation was accompanied by a commensurate stimulation of translation. In other words, do temperatures above the growth optimum stimulate the processes of mRNA translation and degradation equally? Our data suggest that the RNA being degraded does in fact include template RNA and that synchronizing temperatures reduce the functional lifetime of these templates (as measured by net protein synthesis). If one defines, for the purpose of comparison, the efficiency of a messenger RNA (mRNA) pool as the net protein synthesized under a given experimental situation (ideal growth conditions yielding maximum translation and maximum efficiency) it would appear that temperatures above the optimum decrease template efficiency in *Tetrahymena*. Implicit in this interpretation is the assumption that actinomycin D acts uniquely to inhibit RNA polymerase (*5*). Parallel experiments with lipid biosynthesis offer some assurance that this is so, since lipid synthesis is increased during similar temperature elevations and this stimulation is unaffected by the presence of actinomycin D. This would seem to rule out some nonspecific effect of the antibiotic but does not rigorously exclude some undefined deleterious effect apart from polymerase inhibition.

For our experiments *Tetrahymena pyriformis* GL was cultivated in a medium consisting of 0.25 percent proteose-peptone (Difco), 0.1 percent sodium acetate, 0.1 percent dibasic potassium phosphate, and 0.1 percent yeast extract (Difco). The generation time of well-aerated cells in this medium is 6 hours at 29°C. The extent of labeling was assayed by the direct filter-paper-disc method (*6*). Protein synthesis was assayed by the addition of an amino acid mixture uniformly labeled with C^{14} to a logarithmically growing culture (final concentration 3 μc/ml). Lipid synthesis was determined by a slightly modified filter-paper-disc procedure (*7*) with C^{14}-glycerol as the substrate. Glycerol enters almost exclusively into lipids soluble in a mixture of chloroform and methanol under these conditions (*7*). In all cases, the actual assays were done by removing 75-μl portions from the culture (in duplicate) at time zero and at regular intervals thereafter. The cells were placed directly on 2.5-cm discs of Whatman 3MM filter paper, the discs were dropped, while still wet, into cold trichloroacetic acid (5 percent for lipid, 10 percent for protein), and the accumulated discs were washed and processed for scintillation counting (*6*, *7*). Where indicated, actinomycin D was added to a final concentration of 50 μg/ml, approximately twice the concentration needed to suppress uracil incorporation in these cells (*1*). To study the effect of altering the incubation temperature, the cultures were divided into equal portions after addition of the label or antibiotic (or both), and then they were incubated at the temperatures cited.

The effect on protein synthesis of varying the incubation temperature after the addition of actinomycin D is shown in Fig. 1. The strain used here (GL) grows most rapidly at 29°C. It can be brought into synchronous division by cycling the temperature to 34°C

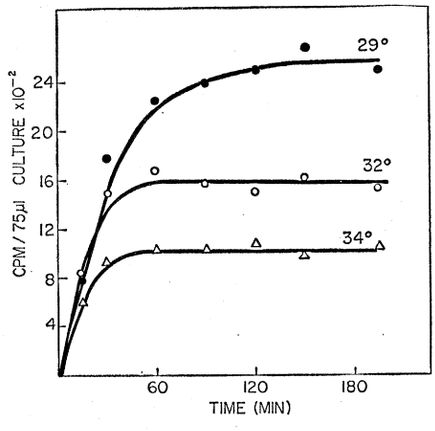

Fig. 1. Incorporation of a C^{14}-amino acid mixture (3 μc/ml) after addition of actinomycin D (50 μg/ml) to a culture of *Tetrahymena pyriformis* GL. Label and antibiotic were added at time zero, after which the culture was divided, and portions were incubated at 29°, 32°, and 34°C. A significant reduction in synthetic capacity occurs as the temperature rises above that optimum for growth (29°C).